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14. ABSTRACT

Increasing evidence from our lab and others support the findings that ribosomal proteins play critical role in development as well as bone marrow disease in addition to its essential role in protein synthesis. We found Rpl22 is dispensable for protein biosynthesis but regulating transformation and hematopoiesis. Previously I had determined that Rpl22 functions as a haploinsufficient tumor suppressor in mouse T-cell lymphoma model by activating the NFκB and its target Lin28B. Recently we also found that Rpl22 knockout mice exhibit MDS-like phenotype associated with anemia and abnormal bone marrow (BM) hematopoiesis.Consistent with what we observed in mouse model, our collaborator found that Rpl22 was mutated or deleted in some MDS and AML patients. With all these data, I intend to investigate the role of Rpl22 in MDS and its predisposition of AML. I found that Rpl22 loss induces Lin28B is specific and the activation of NFκB and Lin28B induction depends on ER stress PERK signaling. By introducing AML oncogenic gene MLL-AF9 into the BM transplant mouse model, we found Rpl22 inactivation accelerates AML progression. We are still in the progress of investigating Rpl22 in MDS/AML and hopefully can find new therapeutic target through these efforts.

15. SUBJECT TERMS

Rpl22, AML, MDS, ER Stress

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1. INTRODUCTION:

Ribosomal proteins (RPs) have been well known to be essential for protein synthesis. Recently increasing evidence from our lab and others support that RP play critical but poorly understood role in development as well as disease. Mutations in RP cause diseases collectively termed ribosomopathies, which is mainly bone marrow diseases such as MDS and is often associated with increased risk for cancer development. We are interested in Rpl22, an RNA-binding component of the 60S ribosomal subunit. Rpl22 is dispensable for protein biosynthesis but regulating transformation and hematopoiesis(1, 3). Previously I have determined that Rpl22 functions as a haploinsufficient tumor suppressor, whose mono-allelic inactivation can accelerate the development of T-cell lymphoma in a mouse model where disease is driven by a MyrAkt2-transgene. Rpl22 inactivation predisposes to transformation by activating the NFκB and its direct target, the stem cell factor Lin28B(3). In addition, we also found that Rpl22 knockout mice exhibit MDS-like phenotype associated with anemia and abnormal bone marrow (BM) hematopoiesis. Consistent with what we observed in mouse model, our collaborator found that Rpl22 was mutated or deleted in some MDS and AML patients. With all these data, I intend to investigate the role of Rpl22 in MDS and its predisposition of AML and hopefully can find out new therapeutic target through these efforts.

2. KEYWORDS: ribosomal protein, Rpl22, MDS, AML, NFκB, ER stress, Lin28B

3. OVERALL PROJECT SUMMARY:

1. *To explore the molecular basis for Lin28B induction by inactivation of Rpl22*

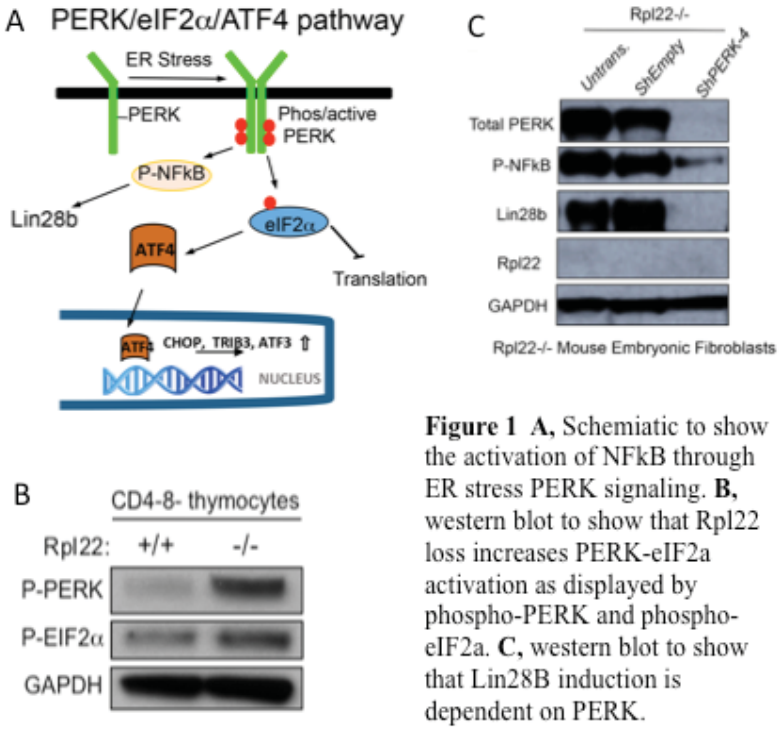

Figure 1 A, Schemiatic to show the activation of NFkB through ER stress PERK signaling. **B,** western blot to show that Rpl22 loss increases PERK-eIF2a activation as displayed by phospho-PERK and phospho-eIF2a. **C,** western blot to show that Lin28B induction is dependent on PERK.

1A: Test whether Rpl22 loss activates NFκB through endoplasmic reticulum stress pathway PERK-phospho-eIF2α-IκBα or IRE1α-TRAF-IKKβ

Recent publications have showed ER stress activation of NFκB signaling(4) and we observed increased ER stress response in Rpl22 loss cells as demonstrated here (Fig.1B) by increased phosphor-PERK and its target phosphor-EIF2α. Therefore, we utilized retroviral-mediated shRNA targeting different ER stress components including PERK, IRE1α, and ATF6 to determine which

signaling pathway is required for Lin28B induction. As shown in Fig.1C, knockdown of PERK significantly abrogate NFκB activation as displayed by phospho-*NFκB* component and Lin28B induction. We found PERK is responsible the activation NFκB activation and Lin28B induction.

1B. Determine whether inactivation of other ribosomal proteins increases Lin28B

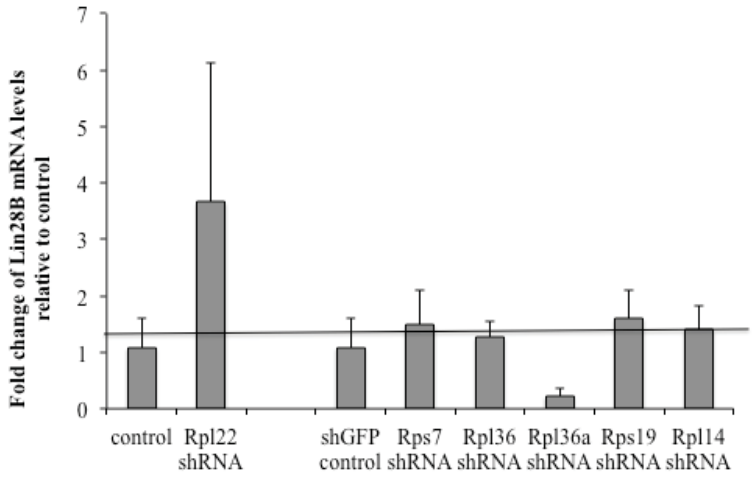

Figure 2 Lin28B mRNA levels were determined by realtime PCR after 72 hours of shRNA infection targeting risbosomal protein Rps7, Rpl36, Rpl36a, Rps19 and Rpl14.

By knockdown other ribosomal proteins with lentiviral-mediated shRNA targeting Rps7, Rpl36, Rpl36a, Rps19 and Rpl14, we failed to observe significant induction of Lin28B as determined by realtime PCR (Fig.2). These data suggest that regulation of Lin28B is specific with Rpl22.

2. *To assess the role of p53 and Lin28B induction in the perturbation of hematopoiesis observed in Rpl22-/- mice*

Ribosomal protein defect always induces p53 induction(5, 6). To assess p53 role in perturbation of hematopoiesis in Rpl22-/- mice, p53 null mice was crossed with Rpl22-/- mice. We start to get p53-/-Rpl22-/- mice for experiments soon. This aim is in progress.

3. *To explore whether Rpl22 loss and Lin28B induction accelerate Myelodysplastic Syndrome /Acute Myelogenous Leukemia progression*

Instead of using AML-ETO9a(7, 8), we used MLL-AF9 another frequent translocation in AML and its oncogenic activity is established in murine model. Mice transplanted with MLL-AF9 cells displayed leukemia phenotype as early as 6 weeks(9). So we transduced BM cells from Rpl22-/- and Rpl22+/+ mice with MLL-AF9 and transplanted the cells into the recipient mice. As we expected, mice receiving Rpl22 KO cells display AML phenotype as shown in Fig.3. Rpl22 loss seems to be predisposed to AML. We are also generating MLL-AF9 transgenic mice with Rpl22 KO to study whether Rpl22 deletion will accelerate AML development *in vivo*.

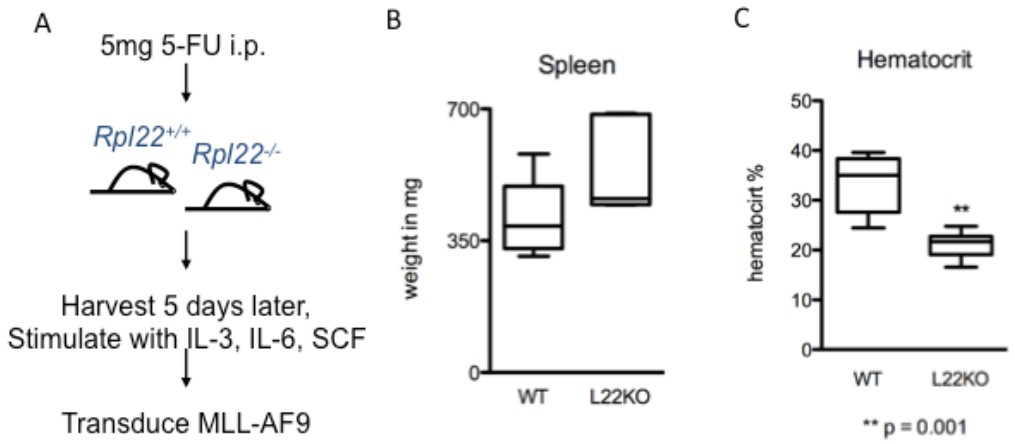

Figure 3 A. Schematic to show the transplant mouse model. BM cells were harvested, transduced with MLL-AF9 oncogene and then transplanted to irradiate recipient mice. B. Spleen weight of the recipient mice receiving transformed BM cells with MLL-AF9. C. Hematocrit of recipient mice receiving BM cells transduced with MLL-AF9.

4. KEY RESEARCH ACCOMPLISHMENTS:

- By investigating the mechanism associated with Lin28B induction caused by Rpl22 defect, we linked inflammation pathway NFκB dependent Lin28B induction with ER stress pathway PERK- *eIF2α*.
- We also found Lin28b induction caused by Rpl22 inactivation is unique as it is not found in other tested ribosomal proteins.
- Most interestingly, as we predicted, Rpl22 loss predisposed mice to AML, suggesting Rpl22 could be a prognosis marker.

5. CONCLUSION:

We found ER stress pathway PERK is responsible for NFκB dependent Lin28B induction caused by Rpl22 defect. Lin28B induction by Rpl22 inactivation is unique, as it is not found in other tested ribosomal proteins. And in mouse transplant model, Rpl22 loss does predispose mice to AML.

6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:

a. **Rao S,** Stadanlick JE, Cai KQ, Wiest DL. 2014 Loss of Rpl22 promotes tumor progression through regulation of angiogenesis and dissemination. AACR-Hematologic Maligancies Conference, Philadelphia, USA
b. Greenberg N, Patel NS, Peri S, **Rao S**, Rhodes M, Wiest DL. 2014 Role of Ribosomal Protein Rpl22 in regulating leukemic transformation. AACR-Hematologic Maligancies Conference, Philadelphia, USA

7. INVENTIONS, PATENTS AND LICENSES: Nothing to report

8. REPORTABLE OUTCOMES:

Rpl22 deletion can predispose mice to AML. Combined with our collaborator's findings that MDS/AML patients with Rpl22 mutations have poor survival rate, we find that Rpl22 inactivation is a potential prognostic marker of progression to AML.

9. OTHER ACHIEVEMENTS:

One travel award from AACR-hematologic malignancies in Sep. 2014.

10. REFERENCES:

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11. TRAINING OR FELLOWSHIP AWARDS:

I joined Dr. David Wiest lab at Fox Chase Cancer Center in 2010. Dr. Wiest's expertise in preclinical animal models of T cell malignancy as well as in the manipulation of development of primary hematopoietic stem cells in vitro and in vivo, has enabled me to significantly expand my technical repertoire and develop a strong interest in hematologic malignancies. Under Dr. Wiest's sponsorship I will focus on the following for additional training, which is necessary for an independent position in academia: 1) Writing Skills – I intend to enroll in a one day NIH grant writing training seminar next Monday at University of Pennsylvania. Moreover, I am in the process of preparing two manuscripts. This will entail several rounds of review and revision with Dr. Wiest, which will also help to improve my writing ability. 2) Oral Presentations – I have won awards for my oral presentations; I seek to further improve my speaking ability through a number of forums: a) weekly Wiest lab meetings; b) the postdoctoral seminar series; c) weekly departmental journal club; d) Fox Chase Research Festival; e) our weekly departmental seminar series where I am able to meet with the outstanding speakers that visit Fox Chase Cancer Center; and f) attending scientific meetings at least once per year. Taken together, these experiences will ensure that I receive a well-rounded training experience and will be fully prepared for independence as a researcher in blood malignancies.

12. APPENDICES

Two abstracts copies and the copies of travel award, see attachment.

a. **Rao S,** Stadanlick JE, Cai KQ, Wiest DL. 2014 Loss of Rpl22 promotes tumor progression through regulation of angiogenesis and dissemination. AACR-Hematologic Malignancies Conference, Philadelphia, USA

b. Greenberg N, Patel NS, Peri S, **Rao S**, Rhodes M, Wiest DL. 2014 Role of Ribosomal Protein Rpl22 in regulating leukemic transformation. AACR-Hematologic Malignancies Conference, Philadelphia, USA

c. One travel award from AACR-hematologic malignancies in Sep. 2014

Moreover, disruption of CXCR4 signaling with a small molecule inhibitor severely impaired T-ALL progression in mouse and human xenograft models of the disease. We found that CXCR4 promotes c-Myc expression, which is required in leukemia initiating cells. Given the relatively subtle role for CXCR4 in normal developing T cells, CXCR4 inhibition to our knowledge has not been proposed as a therapy for T cell malignancies. Our data strongly suggest that targeting CXCR4 could be a promising strategy for combating T-ALL.

A07 Loss of Rpl22 promotes tumor progression through regulation of angiogenesis and dissemination. Shuyun Rao, Jason E. Stadanlick, Kathy Q. Cai, Wiest L. David. Fox Chase Cancer Center, Philadelphia, PA.

Mutations in ribosomal proteins (RP) often cause bone marrow syndromes associated with increased risk for cancer development. We have shown that Rpl22 is a haploinsufficient tumor suppressor in T acute lymphoblastic leukemia (T-ALL). Indeed, loss of one Rpl22 allele accelerates T-cell lymphomagenesis in the murine Akt transgenic mouse model, by inducing the stem cell factor Lin28B in an NFκB-dependent manner. Moreover, mice lacking both Rpl22 alleles exhibited significantly increased thymic tumor size, which was associated with poor survival and markedly enhanced angiogenesis relative to Rpl22 heterozygous mice or wild type. The increased angiogenesis in the mediastinal mass was linked to upregulation of VEGFA and downregulation of S1P1. Loss of S1P1 is sufficient to block egress of lymphoma cells from thymus. Rpl22-deficient thymic lymphoma lines also exhibit enhanced growth upon adaptation to growth in vitro, and this is reversed by ectopic expression of Rpl22. Rpl22 expression also represses the expression of proteins that promote traversal of the cell cycle. Collectively, the increased tumor growth and angiogenesis, and the blockade of thymic egress contribute to the enlarged mediastinal masses observed in Rpl22-deficient mice. Rpl22-mutant T-ALL are a molecularly distinct subtype exhibiting increased aggression and treatment resistance. Accordingly, our identification of a number of signaling pathways controlled by Rpl22 may enable the development of novel strategies, such as targeting angiogenesis, in such cases of T-ALL.

A08 A novel role for the high mobility group A1 (HMGA1) chromatin remodeling protein in mediating AML-niche crosstalk. Stuart A. Rushworth[1], Lingling Xian[2], Kristian M. Bowles[1], Linda M.S. Resar[2]. [1]University of East Anglia, Norwich, United Kingdom, [2]The Johns Hopkins University School of Medicine, Baltimore, MD.

Acute myeloid leukemias (AMLs) are highly lethal hematologic malignancies that arise from diverse genetic abnormalities in hematopoietic progenitors. Unfortunately, most patients with AML will die of their disease due to failure of currently available cytotoxic chemotherapies. Emerging evidence underscores an important role for bone marrow stromal cells (BMSCs) and the bone marrow niche in survival and proliferation of AML blasts. Accordingly, there is an urgent need to better understand the biologic interactions between AML blasts and BMSCs that support their growth and protect them from chemotherapy. This knowledge should inform biologically rational strategies to enhance existing treatments and facilitate the design of novel therapies. Our research focuses on the high mobility group A1 (HMGA1) chromatin remodeling proteins in AML blast survival within the bone marrow niche. HMGA1 proteins regulate gene expression by modulating chromatin structure and recruiting NF-κB and other transcription factor complexes to DNA. HMGA1 gene and proteins are highly expressed during embryonic development. HMGA1 expression is also enriched in embryonic stem cells, diverse solid tumors, and hematologic malignancies, such as AML. Prior studies from our group demonstrate that high expression of HMGA1 correlates with poor outcomes and relapse in leukaemia and solid tumors. We also discovered that HMGA1 regulates transcriptional networks that maintain a stem-like state in diverse tumor types. Here, we uncovered a novel role for HMGA1 in mediating crosstalk between AML blasts and BMSCs within the bone marrow microenvironment. We found that silencing HMGA1 induces apoptotic cell death in AML cell lines. To determine how HMGA1 mediates survival in AML blasts, we assessed expression of pro-survival genes in AML cell lines and primary AML blasts. We found that silencing HMGA1 represses expression of the gene encoding the C-X-C chemokine receptor type 4 (CXCR-4). CXCR-4 is the receptor for stromal cell-derived factor 1 (SDF-1), a growth factor secreted by BMSCs that also serves as a chemo-attractant for hematopoietic stem cells or AML blasts within the bone marrow microenvironment. This led us to hypothesize that HMGA1 regulates crosstalk between AML

B25 Progressive epigenetic programming during B cell maturation yields a continuum of clonal disease phenotypes with distinct etiologies in chronic lymphocytic leukemia. Christopher C. Oakes[1], Marc Seifert[2], Assenov Yassen[1], Lei Gu[3], Martina Przekopowitz[2], Amy Ruppert[4], Andrius Serva[1], Sandra Koser[1], David Brocks[1], Daniel Lipka[1], Olga Bogatyrova[1], Daniel Mertens[1], Marc Zapatka[1], Peter Lichter[1], Hartmut Doehner[5], Ralf Kueppers[2], Thorsten Zenz[6], Stephan Stilgenbauer[5], John Byrd[4], Christoph Plass[1]. [1]The German Cancer Research Center (DKFZ), Heidelberg, Germany, [2]The University of Duisburg-Essen, Essen, Germany, [3]The Broad Institute, Boston, MA, [4]The Ohio State University, Columbus, OH, [5]Ulm University, Ulm, Germany, [6]Nationale Centrum für Tumorerkrankungen (NCT), Heidelberg, Germany.

Knowledge of the cell-of-origin is essential for the full understanding of the causes of a malignant disease and for the rational design of targeted therapies. The B cell compartment is composed of a highly complex mixture of subtypes, each with distinct phenotypes and roles within the immune system. In chronic lymphocytic leukemia (CLL), heterogeneity in the biology and clinical course of the disease is thought to be linked to divergent cellular origins. We and others have previously shown that the epigenome of CLL, as measured by the global pattering of DNA methylation, is highly clonal and remarkably stable over time and thus represents a powerful approach to trace founder subtype populations. Here we combine epigenomic and transcriptomic analysis using next-generation sequencing approaches to compare CLL cells to highly purified and specific B cell subpopulations at various stages of maturation. We find that B cell maturation involves substantial unidirectional epigenetic programming that occurs as a continuum throughout the transition between naïve to fully-mature memory B cell subpopulations. Combining 258 CLL cases using Illumina 450K analysis reveals that all CLLs arise from a discrete window within the spectrum of B cell maturation that is more similar to mature B cells, with the majority of cases clustering at two distinct points correlating with unmutated IGHV versus highly mutated (<95% homology) IGHV genes; however, a significant number (~20%) of cases arise at various points between these two clusters. Next we show that using RNA-seq, broad differences in global expression patterns mirror the degree of epigenetic programming achieved by individual CLLs. Progressively further programming is paralleled by a transition from an aggressive to indolent expression pattern, indicated by the decrease in the expression levels of genes with known roles in promoting CLL cell survival, such as ZAP70, BTK, TCL1a, MCL1, miR-155 and others. Using DNA methylation and ChIP-seq data to compare the sequence and chromatin features of genomic regions that are programmed in normal B cell maturation versus CLL, reveals that although a myriad of transcription factors and pathways connected to immune cell function are involved in normal epigenetic programming in B cells, aberrant CLL-specific alterations involve excess activity of NFAT and EGR gene families and, paradoxically, a reduction of AP-1 activity. To further investigate the role of immediate-early genes, RNA-seq analysis of in vitro-activated CLL cells revealed a strong association between the degree of epigenetic programming and the specific inducibility of EGR2 and c-FOS, supporting a functional role of these genes in aberrant DNA methylation programming. Finally, in an independent clinically well-annotated cohort of 349 CLL cases, we demonstrate that the degree of epigenetic programming is significantly associated with time to treatment and overall survival in patients. Collectively, this work demonstrates that instead of a distinct cell(s)-of-origin, CLL is rather derived from a continuum of possible programming states, and that the degree of programming achieved by a particular CLL at the time of transformation dictates its global gene expression pattern and clinical outcome. Furthermore, a parallel assessment of B cell maturation with CLL development permits a refinement of the disease-specific, early molecular events, highlighting the dysregulation of particular transcription factors and pathways in the pathogenesis of aggressive versus indolent disease.

B26 Role of ribosomal protein, Rpl22 in regulating leukemic transformation. Noa Greenberg, Nehal Solanki Patel, Suraj Peri, Shuyun Rao, Michele Rhodes, David Wiest. Fox Chase Cancer Center, Philadelphia, PA.

Inactivation of ribosomal proteins (RP) is known to cause diseases called ribosomopathies, which are often associated with abnormal hematopoiesis and an increased risk for development of leukemia. Our laboratory has recently reported an important link between inactivation of one such ribosomal protein, Rpl22, and poor survival in T cell acute lymphoblastic leukemia (T-ALL) patients. Preliminary bioinformatic analysis shows that

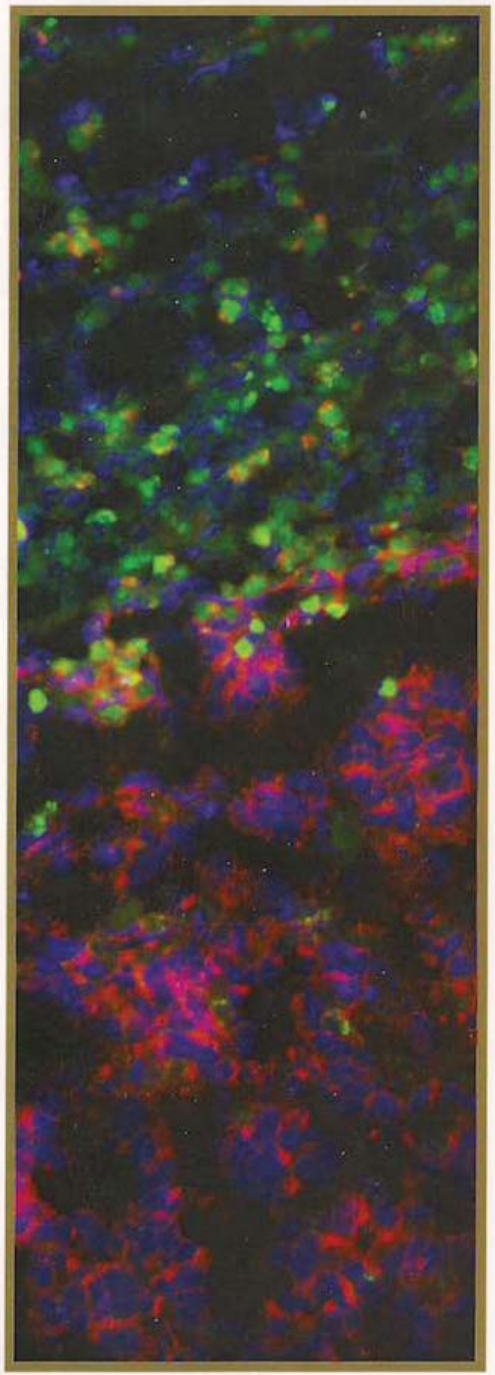
RPL22 is lost in ~10% of pediatric T-ALL patients, who exhibit a more aggressive disease course. Deletion of the RPL22 locus is also enriched in the early T-cell precursor (ETP-ALL) subset of T-ALL patients, which exhibit a more aggressive disease course. Thus, RPL22 loss appears to be a marker for poor outcome in pediatric T-ALL patients. While RP inactivation has previously been linked to increased cancer risk, the mechanistic basis for this linkage remained unclear. We have recently demonstrated that inactivation of Rpl22 promotes leukemic transformation by activating NF-kB and inducing the stem cell gene, LIN28B. Nevertheless, the molecular link between Rpl22 inactivation and the induction of NF-kB activity remained unclear. We have now determined that inactivation of Rpl22 activates NF-kB signaling by exacerbating ER stress responses. Indeed, Rpl22 loss results in hyperactivation of the PERK-EIF2α-ATF4 stress pathway, which is known to activate NF-kB. We have demonstrated that knockdown of PERK using shRNA abrogates the activation of NF-kB in Rpl22 mutant cells and returns Lin28B expression levels to baseline. This reduction in Lin28B eliminates the predisposition to transformation exhibited by Rpl22 mutant cells, as evidenced by decreased formation of colonies in soft agar. Taken together, these data suggest that RPL22 inactivation may serve as a negative prognostic indicator in T-ALL and that pharmacologic targeting of ER stress pathways may represent a novel therapeutic alternative in this molecularly-defined subclass of T-ALL.

B27 Epigenetic regulation of stem cell fate in leukemic subpopulations. Hsing-Chen Tsai, Hariharan Easwaran, Stephen B. Baylin. Johns Hopkins School of Medicine, Baltimore, MD.

Leukemia often displays substantial intratumor heterogeneity, comprising subpopulations of cells with different self-renewing and leukemia initiation capacities. Epigenetic mechanisms have been implicated in stem cell fate regulation during normal hematopoiesis. Although aberrant epigenetic changes have been well documented in hematological malignancies, the role epigenetic mechanisms play in governing stem-like properties, such as leukemia initiating capacity, and possible cell fate transitions between stem-like and non-stem-like leukemia subpopulations is unclear. Using next-generation sequencing and microarray technologies, we conducted an integrated analysis of DNA methylation, histone modifications and transcriptional profiles to investigate the epigenomic differences between experimentally characterized CD34-positive (CD34pos) leukemia-initiating and CD34-negative (CD34neg) non-leukemia-initiating subpopulations in a human acute myelogenous leukemia (AML) cell line. We demonstrated that multiple normal and leukemic stem cell gene signatures are significantly enriched in CD34pos leukemia initiating cells as opposed to CD34neg AML cells. We also observed concordant chromatin state transitions at the promoters of stem cell signature genes. The stem-like state of CD34pos leukemia initiating cells is also characterized by a number of genes with bivalent histone modifications at their promoters. Many genes undergoing bivalent domain resolution and transcriptional activation in CD34neg AML cells are involved in differentiation-related pathways. Most importantly, epigenomic analysis showed that treatment with epigenetic modifying agents, such as 5-aza-2'-deoxycytidine, appeared to facilitate cell fate transitions, induce marked changes in chromatin configurations, and reverse the enrichments of stem cell signatures in CD34pos leukemia-initiating cells. This provides an important molecular basis for the diminished in vitro clonogenecity and in vivo leukemogenecity of AML cells after 5-aza-2'-deoxycytidine treatment in our previous studies. Our data supports the important role of epigenetic mechanisms in underpinning cell fate and functional properties of leukemia subpopulations. The epigenetic regulation of leukemic stem-like properties might differ from that of normal hematopoietic stem cell state since the same 5-aza-2'-deoxycytidine treatment did not seem to negatively affect clonogenecity of normal hematopoietic cells. Understanding the dynamics of epigenetic state transitions between leukemia subpopulations may offer novel targets for leukemia treatment, and facilitate development of epigenetic therapy targeting leukemia initiating cells.

B28 Co-occupancy of AML1-ETO and N-CoR defines a dominant phenotypic signature in leukemic cells. Daniel J. Trombly[1], Troy W. Whitfield[1], Srivatsan Padmanabhan[1], Jonathan A. Gordon[2], Jane B. Lian[2], Andre J. van Wijnen[3], Sayyed K. Zaidi[2], Janet L. Stein[2], Gary S. Stein[2]. [1]UMass Medical School, Worcester, MA, [2]University of Vermont, Burlington, VT, [3]Mayo Clinic, Rochester, MN.

The t(8;21) chromosomal translocation produces AML1-ETO, an oncogenic fusion protein that compromises the function of AML1, a transcription



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